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Review Paper

Daughters on request: about helpers and egg sexes in the Seychelles warbler

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The Seychelles warbler (*Acrocephalus sechellensis*) was an endangered endemic of the Seychelles islands where, until 1988, the entire population of *ca*. 320 birds was restricted to the one island of Cousin Island (29 ha). Additional breeding populations were successfully established on the islands of Aride (68 ha, 1988) and Cousine (26 ha, 1990) and now with the existence of *ca*. 2000 warblers on three islands the conservation status of the warbler has improved from endangered to vulnerable. Emigration from the island is extremely rare, so birds that disappeared were known to have died. Almost every bird on Cousin Island has been individually colour ringed and monitored throughout all breeding attempts during a 17-year period (1985–2002; total *ca*. 2400 birds). These birds were also blood sampled for molecular parentage and sex analyses. Therefore the lifetime reproductive success of many birds is known. Although warblers can breed independently in their first year, some individuals remain in their natal territory as subordinates, and often help by providing nourishment to non-descendant offspring. The frequency of 'helping' is affected by habitat saturation and variation in territory quality (insect prey availability). The long-term benefits of helping are higher for daughters than for sons, and it is therefore no wonder that most helpers are daughters from previous broods. Furthermore, on low-quality territories breeding pairs raising sons gain higher fitness benefits than by raising daughters, and vice versa on high-quality territories. Female breeders adaptively modify the sex of their single-egg clutches according to territory quality: male eggs on low quality and female eggs on high quality. The Seychelles warbler is a beautiful example of behavioural and life-history adaptations to restricted circumstances.

Keywords: Seychelles warbler; cooperative breeding; conservation; sex allocation; sex determination; fitness

1. INTRODUCTION

In contrast to most invertebrates, the young of many vertebrate species require a long period of parental care before reaching independence. In birds, the young are sometimes raised by either the male or the female, but usually by both parents. However, there are examples of mature individuals delaying independent breeding and instead providing care to young that are not their own genetic offspring. In birds, these helpers usually assist with feeding nestlings or fledglings, but also with incubation and defence of the nest or territory. Sometimes individuals spend all of their lives helping others to reproduce. Such systems of cooperative breeding are known to occur in only 3.2% of extant bird species (e.g. Sibley & Monroe 1990; Arnold & Owens 1998). Although the existence of helpers has been known for many years (Skutch 1935), it was not until Hamilton (1964) and Maynard Smith (1964) developed the theory now referred to as 'kin selection' that there was a firm foundation for the empirical study of cooperative breeding. These authors argued that the fitness of each individual is determined by the total number of genes, identical by descent to its own, that are present in following generations. As such, helpers can increase their fitness by gaining either indirect fitness benefits from enhancing the reproductive success of close relatives, or direct benefits from increased opportunity for their own future reproduction (e.g. Emlen & Wrege 1989; Mumme *et al.* 1989; Emlen 1991, 1995; Koenig *et al.* 1992; Mumme 1992; Cockburn 1998). However, in most species the fitness benefit from helping is likely to be considerably less than the potential direct genetic gain from immediate independent breeding if a territory and mate could be obtained (Brown 1987). Thus cooperative breeding can be seen as a 'best-of-a-bad-job' strategy, adopted when opportunities for independent breeding are limited. Therefore, understanding why grown offspring delay dispersal is the key to understanding the evolution of cooperative breeding. The usual approach is to focus on the 'ecological constraints', such as shortage of breeding territories or mates, which prevent offspring from becoming independent breeders (Selander 1964; Emlen 1982, 1991; Brown 1987; Koenig *et al.* 1992). However, another approach is to focus on the benefits gained by remaining on the natal territory (Stacey & Ligon 1987, 1991; Waser 1988). In other words, if variation in habitat quality increases the benefits of philopatry, cooperative breeding can be found in the absence of territory shortage ('ecological constraints'). Because both perspectives were seen as different hypotheses, the relative merits of these ideas were the subject of a lively debate in the 1980s. At that time, in December 1985, I started my research on the Seychelles warbler (*Acrocephalus sechellensis*) on the small island of Cousin (28 ha) in the Seychelles, Indian Ocean.

I chose to study this species because little was known of its cooperative breeding system at that time (Diamond

1980). To conduct research on an uninhabited tropical island seems ideal. First, I had no direct access to any university library and was unaware of the discussions on cooperative breeding. This enabled me to develop my own ideas, which were continually nurtured from careful observations and experiments in the field. Second, originally being entirely confined to Cousin Island, I was able to study the entire world population of Seychelles warblers. The Cousin population of warblers has been intensively monitored between December 1985 and September 2002, and the populations on the islands of Aride and Cousine, which were established in 1988 and 1990, respectively, were studied from that time onwards. Each year nearly all unringed warblers were caught and ringed with a unique combination of colour rings (two colour rings on one leg and one colour ring and an individually numbered British Trust for Ornithology ring on the other leg). This resulted in 96% of the Cousin population (*ca*. 320 birds) being individually recognizable. I traced all dispersing individually marked birds and monitored their breeding activity throughout their lives. An additional advantage, which I was unaware of when I began my study, was the unique opportunity to calculate the long-term benefits of cooperative breeding for the helper and the breeding pair. With the use of powerful molecular genotyping techniques developed in the early 1990s, it has been possible to accurately determine parentage from a tiny blood sample (*ca*. 15 μl) collected by brachial venipuncture. Since 1993, almost the entire adult population and all the young have had their blood sampled each year. This has allowed the precise calculation of the total number of young produced over the entire lifetime of helpers and both members of the breeding pair. The molecular techniques yielded additional advantages, such as sex determination of newly hatched young and determination of relatedness within cooperative breeding units, which allowed the expansion of the scope of the research. Over the course of time many people collaborated in the research on the Seychelles warbler and enabled me to write this review.

In this review, I will first describe the actions taken to conserving the threatened Seychelles warbler. Second, I will present some major findings of the long-term research. I will address the causes of cooperative breeding, the fitness consequences of cooperative breeding for the helper and their parents, and finally I will address some further adaptations employed by helpers and parents to enhance their fitness.

2. CONSERVING A THREATENED SPECIES

The Seychelles warbler, closely related to the migratory great reed warbler (*Acrocephalus arundinaceus*) and Australian reed warbler (*A. australis*) (Leisler *et al.* 1997), is endemic to the Seychelles Islands in the Indian Ocean and was entirely restricted to Cousin Island (figure 1*a*,*b*). It is a small (15–16 g) insectivorous bird that gleans invertebrate prey from leaves (figure 1*c*). Once paired, warblers reside permanently in the same territories, sometimes for as long as 9 years. On Cousin Island the warbler usually has a clutch size of one egg only, and adult birds have high annual survival (81%). Young birds require about three months of parental food provisioning (Komdeur 1996*a*). In the 1870s the warbler was recorded on the islands of Mahé (154 km^2), Marianne (74 ha), Félicité (140 ha), Cousine (26 ha) and Cousin (Oustalet 1878; Diamond 1980; figure 1*a*). It is assumed that the Seychelles warbler originally occurred on most of the Seychelles islands, as they constituted a single large island during the last Ice Age (Collar & Stuart 1985). Between 1910 and 1920, the islands were planted with coconuts (*Cocos nucifera*) leaving very little suitable natural habitat for the warblers, resulting in its disappearance from all islands, except Cousin (Collar & Stuart 1985) where by 1967 only *ca*. 30 individuals remained (Crook 1960; Loustau-Lalanne 1968). Because the warbler was threatened with extinction, Cousin was purchased in 1968 by a consortium including Christopher Cadbury, the Royal Society for Nature Conservation and BirdLife International (then the International Council for Bird Preservation) with the express aim of saving the warbler. The coconuts were cleared and the native vegetation was allowed to regenerate resulting in the warbler staging a dramatic recovery (figure 1*e*). By 1982 the population had grown to nearly 320 birds. Since that time, numbers of birds have fluctuated around the same level, indicating that the warbler population has reached carrying capacity. The increase in number of territories showed the same trend and reached its saturation level of *ca*. 115 territories in 1981 (figure 1*e*). In each of the years from 1981 until 2001, there has been a surplus of adult birds resident on the island with *ca*. 320 mature birds for *ca*. 115 territories (Komdeur 1992; Richardson *et al.* 2001; figure 1*e*). The island was completely covered by territorial groups with no empty spaces (figure 1*d*).

Seychelles warblers do not differ from other closely related migratory *Acrocephalus* warblers in the anatomy of the flight apparatus (wing shape, wing loading, skeletal parts and musculature) (Komdeur *et al.* 2003). Despite the potential of sustained flight, the over-production of adult warblers on Cousin in the last decades, and the small distance between Cousin and Cousine, inter-island dispersal was extremely rare. During the 17 years of study only two warblers (0.13%, $n = 1599$) have crossed the sea between the islands of Cousin and Cousine unaided (Komdeur *et al.* 2003). It seems that the Seychelles warbler has a behavioural reluctance to cross what it may regard as extensive bodies of water. Given the vulnerability of a single isolated population, and the extremely small chance of successful establishment on unoccupied islands by unaided warblers, it was decided to translocate birds from Cousin to the islands of Aride and Cousine by motorboat. In September 1988, 16 males and 13 females were transferred to Aride and in June 1990, 15 males and 14 females were transferred to Cousine (Komdeur 1994*a*, 1997). All birds survived the transfer and swiftly accepted their new habitats. Within a few weeks of the transfers most birds had paired, established territories and initiated nesting activities. Some pairs started successful nesting within 3 days of release. The first young hatched three weeks after the transfers. On the new islands, with significantly more food available per territory than on Cousin, I observed a twofold increase in clutch size (Komdeur 1996*a*). Despite the existence of a narrow seasonal window for successful reproduction on Cousin, breeding on the new islands also took place successfully outside this window (most birds bred year-round; Komdeur 1996*a*).

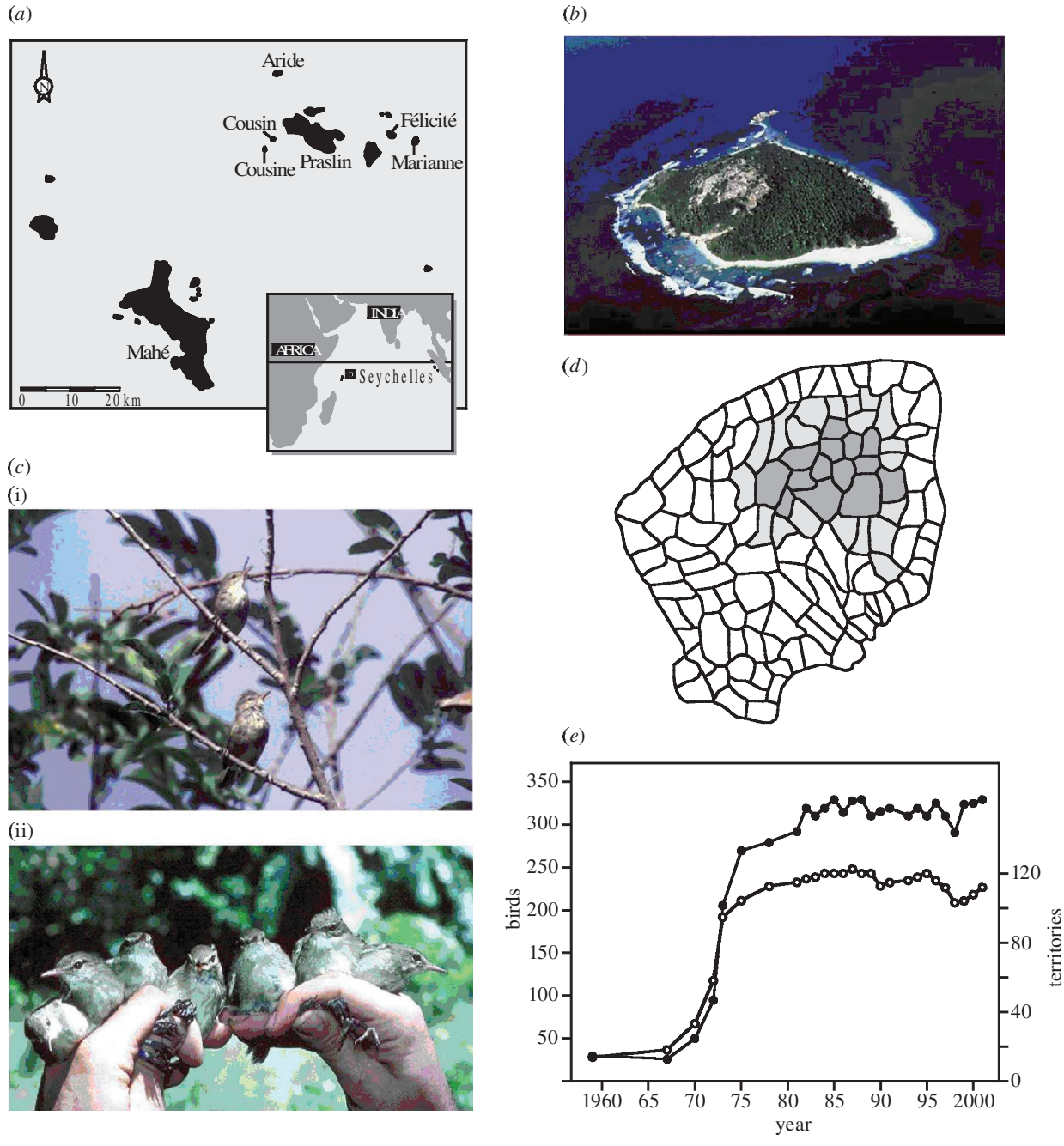

Figure 1. (*a*) Map showing the Seychelles islands. Populations of Seychelles warblers on Cousin, Aride as place of first transfer (September 1988), and Cousine as place of second transfer (June 1990). (*b*) Aerial photograph of Cousin island (photograph: J. Komdeur). (*c*)(i) A pair of Seychelles warbler (photograph: J. Komdeur) and (ii) a complete breeding group of Seychelles warblers on high-quality territory: a breeding pair, three helpers and their offspring (twins) (from left to right: male (1 year, twin), mother (7 years), female helper (4 years), female helper (2 years), female (1 year, twin), father (6 years) and female helper (2 years); photograph: D. S. Richardson). (*d*) Map of Cousin Island with Seychelles warbler territories divided into three quality categories: white, low; light grey, medium; and dark grey, high. (*e*) The increase in Seychelles warbler numbers (black circles) and territories (white circles) on Cousin Island since 1959.

Reproduction was not constrained by moult; birds were able to moult and breed at the same time (Komdeur 1996*a*). On Cousin, however, birds undergo a pre-breeding moult (Komdeur 1996*a*). During the years following the translocation, with high food availability year-round, warblers on the new islands had significantly higher reproductive success than birds remaining on Cousin. By 1997, the Cousine population had increased to 186 individuals (Komdeur *et al.* 1998), and the Aride population to *ca.* 1600 individuals (Cuthbert & Denny 1997). By 2002 both populations appeared to have reached carrying capacity (D. S. Richardson, personal communication). The conservation status of the warbler has improved from endangered to vulnerable (Richardson 2001).

3. COOPERATIVE BREEDING

Since 1973 young, but sexually mature birds in insect-rich territories on Cousin Island remained on their natal territories and assisted their parents to raise subsequent

Table 1. Demographic characteristics of the Seychelles warbler on Cousin Island (from Komdeur 1992). (Numbers in brackets indicate sample size.)

	territory quality		
	low	medium	high
annual number of yearlings produced			
unaided pairs	0.19 (286)	0.51 (38)	0.85 (28)
pairs with one helper	0.22 (49)	0.85 (12)	1.62 (13)
annual survival			
first-year birds	30% (103)	67% (23)	86% (22)
adults	76% (156)	88% (64)	91% (48)
percentage of yearlings that delay dispersal	29% (41)	69% (26)	93% (38)

broods (Diamond 1980). These young became helpers at a time when vacant areas of lower-quality habitat were still present on the island where they could have established a territory and reared a family of their own (figure 1*d*). Apparently, habitat saturation alone was not the main cause of cooperative breeding. The amount of insect food present in a territory could also be an important factor. I measured territory quality as the estimated number of leaf insects present in a territory (Komdeur 1992), and divided territories into three categories of quality: low, medium and high. There was marked variation in territory quality, with the lusher insect-rich territories in the centre of the island and the poor territories on the coast (figure 1*d*). Territory quality was positively correlated with survival and reproductive success (table 1). As a consequence, fewer vacancies arose on high-quality territories (table 1). The options for young warblers were either to fill a vacancy in a low-quality territory, where the probability of immediate breeding was higher, or to remain as a non-breeder in a territory of higher quality. Furthermore, fitness pay-offs from either dispersing or staying and helping had to be considered for each territory quality. Warbler pairs without helpers occupying high-quality territories produced, on average, 0.85 yearlings per year, but pairs in low-quality territory produced only 0.19 yearlings. A young warbler that remains on its high-quality natal territory as a helper enhances the reproductive success of its parents from 0.85 to 1.62 yearlings per year. The presence of a helper resulted in 0.77 extra young for the breeding pair. On average, birds remaining and helping in high-quality natal territory gained more than four times the progeny than when dispersing and breeding independently in a low-quality territory (table 1). The helping benefits were confirmed by helper removal experiments (Komdeur 1994*a*). By contrast, first-year birds and helpers on low-quality territories did better to fill a low-quality vacancy immediately (table 1). My data supported this prediction. One-year-old warblers from high-quality territories were more likely to remain on their natal territory as helpers than were yearlings from medium- or high-quality territories (table 1).

Thus, observational data indicated that the frequency of delayed dispersal and cooperative breeding in the Seychelles warbler is influenced by both habitat saturation (ecological constraints) and variation in territory quality (benefits of philopatry). To examine the relative importance of these factors I performed a translocation experiment. Seychelles warblers from Cousin were captured and translocated to two nearby but unoccupied islands, Aride (68 ha; 9 km north of Cousin) and Cousine (1.6 km southwest of Cousin) (figure 1*a*). These islands had a significantly greater food supply than Cousin (Komdeur *et al.* 1991; Komdeur 1994*b*) and had been suitable for warblers for at least 25 years since the original habitat recovered after the demise of the coconut plantations in the 1960s (Garnett 1977).

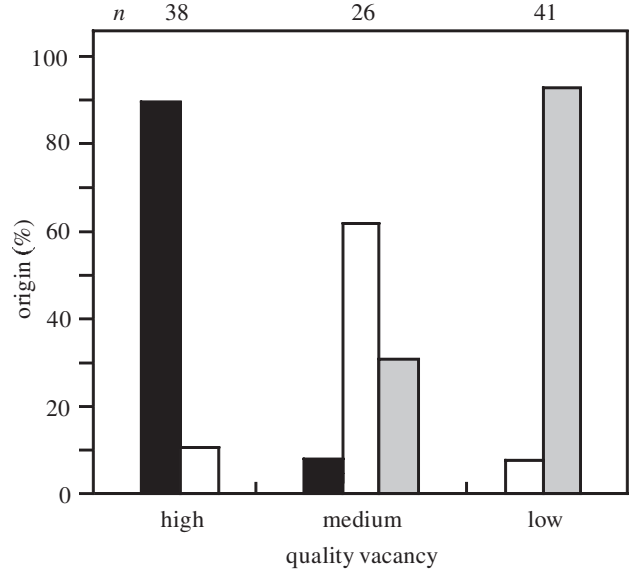

Figure 2. The effect of quality of breeding vacancies on Cousin Island on the origin of individual Seychelles warblers that filled the vacancies (*n*, number of vacancies; from Komdeur 1992). Mean quality territory: grey bars, low; white bars, medium; black bars, high.

After the translocations of birds to the islands of Aride and Cousine I was looking for the answers to the following two main questions. (i) How long would it take to fill the vacancies created on Cousin and which birds would fill them? (ii) What would the young birds born on the new islands do? Would they remain on their natal territories to help their parents, or would they leave at an early stage to establish a high-quality breeding territory elsewhere? The results were dramatic and unambiguous. First, the 38 vacancies created on Cousin were filled immediately (some within hours) by formerly non-breeding helpers, which had dispersed from territories of the same or lower quality (figure 2). In other words, non-breeders from high-quality territories had dispersed to fill vacancies on other high-

quality territories, but did not fill medium- or low-quality vacancies. Similarly, non-breeders from medium-quality territories never filled vacancies on low-quality territories (figure 2; Komdeur 1992). These results clearly demonstrate that dispersal decisions by warblers are influenced by the relative quality of both the natal and vacant territory.

Second, all of the offspring initially produced by translocated birds on 'unsaturated' Aride and Cousine had dispersed from their natal territories as yearlings, and none became helpers ($n = 105$) (Komdeur 1992). Thus, in the absence of habitat saturation, delayed dispersal and cooperative breeding simply did not occur. Only later, when all of the high-quality areas on Aride and Cousine became occupied, did young birds from the best territories begin to remain on their natal territories and act as helpers. This occurred, even though there was abundant space in lower-quality areas to establish territories (Komdeur 1992; Komdeur *et al.* 1995). These results demonstrate clearly that the expression of delayed dispersal in the Seychelles warbler is influenced by habitat saturation ('ecological constraints') and variation in territory quality ('benefits of philopatry'). These results provided a strong experimental confirmation of two previously much-debated hypotheses about the factors influencing delayed dispersal and group augmentation. However, why subordinates then help is still much debated (Emlen 1995; Arnold & Owens 1998; Cockburn 1998; Hatchwell & Komdeur 2000). Helping is not an inevitable consequence of group living, because non-helping subordinates occur in many cooperative societies (Cockburn 1998), including the Seychelles warbler. Furthermore, philopatry and helping is often biased with respect to offspring sex (Brown 1987; Stacey & Koenig 1990; Emlen 1991; Cockburn 1998). In the Seychelles warbler most helpers were female (88%, $n = 217$; Komdeur 1996*b*), indicating that females may obtain more benefits from helping than males.

4. MUTUAL BENEFITS OF COOPERATIVE BREEDING

(a) *Benefits to subordinates: difference between the sexes*

Indirect fitness benefits have been suggested to be a major selective force behind the evolution of cooperative breeding (Emlen & Wrege 1989; Mumme *et al.* 1989; Mumme 1992; Koenig *et al.* 1992; Emlen 1997). These benefits accrue if helping obeys two requirements. First, helping should result in improved survival and reproductive success of the breeding pair. Evidence that helpers generally do enhance breeder fitness is well documented (reviewed in Emlen 1991; Cockburn 1998). Also in the Seychelles warbler, I have shown experimentally that helping behaviour significantly improved reproductive success of the breeding pair (Komdeur 1994*a*). Second, subordinates should preferentially help more closely related kin. Some studies, the Seychelles warbler included, found that non-breeders were more likely to become helpers and provided more help when they were more closely related to the recipient (Clarke 1984, 1989; Curry 1988; Emlen & Wrege 1988, 1989; Komdeur 1994*c*; reviewed in Emlen 1997; Komdeur & Hatchwell 1999). However, in these studies relatedness was estimated from pedigree data, which can be inadequate because complex patterns of shared reproduction and/or extra-group paternity may be overlooked. Until recently, accurately determining relatedness within complex cooperative breeding systems was fraught with problems (e.g. McRae & Amos 1999; but see Parker *et al.* 1999). The advent of powerful molecular genotyping techniques (Queller *et al.* 1993) means that we are now able to quantify the indirect benefits accruing to subordinates within cooperatively breeding species. With the recent development of microsatellite markers to calculate precisely coefficients of genetic relatedness between individuals in the Seychelles warbler (Richardson *et al.* 2000), we were able to test whether subordinates preferentially help more closely related relatives. To our knowledge, this has not been demonstrated before in cooperatively breeding birds. In the Seychelles warbler, female subordinates without parentage accurately maximized their indirect benefits by preferentially feeding more related offspring, produced by relatives of the female subordinate. However, the amount of help provided by male subordinates was low and independent of relatedness to offspring (Richardson *et al.* 2003).

There are, however, many examples of cooperative systems where unrelated birds commonly help (helpers born in other groups; e.g. Reyer 1980; Emlen 1982; Vehrencamp 1983; Sherley 1990; Whittingham *et al.* 1997; Cockburn 1998; Hatchwell & Komdeur 2000). Therefore, these studies have led to the suggestion that direct benefits by which helpers increase their future reproduction are important (Koenig *et al.* 1992; Cockburn 1998). First, I wanted to test whether helping improved parental performance. For the translocation of warblers to the islands of Aride and Cousine I selected even-aged males and females with different degrees of previous breeding experience: (i) experienced breeders that had fledged young of their own in a previous year; (ii) experienced helpers that had no breeding experience but only helping experience; and (iii) inexperienced birds that had neither helping nor breeding experience. On the new islands (Aride and Cousine), birds with helping experience that were paired with an experienced partner produced their first fledgling as fast as experienced breeders, and significantly faster than inexperienced birds, paired with an experienced partner. Females with helping experience or breeding experience built better nests and spent more time incubating than inexperienced females. During this period no helpers assisted the breeding pair in raising broods. However, for pairs comprising a male with breeding experience and an inexperienced female it took four times longer to produce their first fledgling than for pairs consisting of a female with breeding experience and an inexperienced male (Komdeur 1996*c*). This is because only females build nests and incubate the clutch. In this study, environmental effects were minimized, as comparisons were made between the reproductive success of breeding pairs occupying the same quality territories. Once inexperienced birds had fledged young, and thus had acquired breeding experience, they subsequently improved their breeding success by producing a second fledgling in the same time interval as birds with either helping or breeding experience (Komdeur 1996*c*). I concluded that helping translates into improved reproductive success when a helper becomes a breeder. Although at that time I was unable to determine

parentage of these helpers, I was convinced that some female helpers became co-breeders within their social group through jointly laying eggs with their mother (Komdeur 1991, 1999). A decade later, when the microsatellite-based genotyping became available, we found that female helpers often gained direct benefits through parentage within their own group by sneaking their own eggs into their mother's nest (44% of female subordinates; Richardson *et al.* 2001, 2002). Subordinate males also gained direct benefits through parentage within the group (15% of male subordinates) though significantly less than females (Richardson *et al.* 2002). Direct breeding benefits were found to be significantly higher than indirect kin benefits for both female and male subordinates (Richardson *et al.* 2002). This indicates that parenting skills could be acquired as a result of direct breeding experience within their own group rather than through helping experience.

(b) *Benefits to the breeding pair: adaptive manipulation of egg's sex*

The above results show that, as predicted, females remaining on the natal territory as helpers gain greater benefits than male helpers and this may explain why most helpers are female. However, the story does not end here. Given that helpers on high-quality territories increase their parents' reproductive success (Komdeur 1994*a*), it may be that primary birds on high-quality territories preferentially accept or produce female subordinates to increase their fitness. However, given that helpers on low-quality territories reduce their parents' reproductive success (Komdeur 1994*a*) females on low-quality territories may preferentially expel females or produce males, which disperse in order to avoid having future helpers. Helpers are mostly females, and we have previously suggested that females (in birds females determine the sex of an offspring because they are the heterogametic (ZW) sex) may adjust the sex of their single egg to territory quality (Komdeur 1996*b*). However, at that time I was unable to sex young warblers at hatching. Later this problem was solved with the advent of simple molecular sex identification techniques using DNA extracted from a small blood sample (e.g. Griffiths 1992; Griffiths *et al.* 1992, 1996; Griffiths & Tiwari 1993; Lessells & Mateman 1996, 1998).

Reviews from the 1980s were unanimous in the belief that facultative adjustment of offspring sex ratio at birth was unlikely to occur in vertebrates (Williams 1979; Charnov 1982; Clutton-Brock 1986; Bull & Charnov 1988). Sex determination is almost ubiquitously associated with chromosome heterogamety, constraining the physiological or genetic mechanisms for skewing the sex ratio at birth (Williams 1979, 1992; Krackow 1995). The occurrence of adaptive sex ratio manipulation at laying in birds has, therefore, been questioned. At the time we were able to demonstrate that Seychelles warblers adaptively modify the sex of their single egg. The fraction of male eggs produced by unassisted Seychelles warbler mothers changed significantly with territory quality (Komdeur 1996*b*; Komdeur *et al.* 1997; figure 3). Unassisted females on high-quality territories produced 88% female eggs ($n = 32$), whereas unassisted females on low-quality territories produced 77% male eggs ($n = 57$), the dispersing sex (Komdeur *et al.* 1997). This was confirmed by experiments. (i) Helper removal experiments confirmed that sex-ratio bias was for the purpose of producing helpers. When females on high-quality territories had their female helpers experimentally removed they switched from producing all sons to producing 83% females (Komdeur *et al.* 1997). (ii) Breeding pairs that were transferred from low- to high-quality territories switched from the production of male to female eggs (Komdeur *et al.* 1997). Virtually nothing is known about the mechanisms of egg sex modification. With female heterogamety in birds, adjustment of the clutch sex ratio could arise either before or after ovulation. Pre-ovulation control could occur through segregation distortion of sex chromosomes at the first meiotic division or through sex-biased release of ova from the ovary (Ankney 1982; Krackow 1995, 1999; Oddie 1998). Post-ovulation control could operate through sex-selective re-absorption of the ova in the oviduct or dump laying eggs of the 'unwanted' sex (Emlen 1997). The key difference between pre- and post-ovulation mechanisms of adjustment is that post-ovulation control presumably requires bypassing a day when an egg could have been laid. Typically, warblers produce only single-egg clutches, but by translocating pairs to a vacant habitat of very high quality on Aride Island, most females were induced to produce two-egg clutches without laying gaps. Overall, during the period 1988–1991, females on Aride Island skewed their clutch sex ratios towards daughters (87%, 86 clutches; Komdeur *et al.* 2002). This bias was evident in the first egg (71%), but critically, also in the second eggs laid one day apart, even when all absent, unhatched or unsexed second eggs were assumed to be male (64%). The sex ratio skew observed in the second egg could only arise through pre-ovulation control (Komdeur *et al.* 2002).

In the short term, the manipulation of the offspring's sex is directly adaptive from the perspective of the breeding pair. The next step was to determine whether egg sex-ratio manipulation resulted in long-term inclusive fitness benefits (Komdeur 1998). I undertook an experiment during 1994 to 1996 by selecting unassisted breeding pairs on low- and high-quality territories that were feeding a nestling of the adaptive sex. Through swaps of nestlings immediately after hatching, some breeding pairs were forced to raise a foster-son and some to raise a foster-daughter, so that the subsequent inclusive gains could be compared for foster pairs raising the less adaptive sex and the adaptive sex. Inclusive fitness was estimated as the sum of estimated fitness obtained through the breeding offspring (grandchildren) and the fitness obtained through the helping offspring (in the form of extra offspring produced by the breeding pair through help). On low-quality territories breeding pairs raising foster-sons gained significantly higher inclusive fitness benefits than by raising foster-daughters, and vice versa on high-quality territories with breeding pairs raising foster-daughters (Komdeur 1998). There is good experimental evidence that the inclusive fitness consequences of sex allocation in the Seychelles warbler are adaptive for the breeding pair. However, given the occurrence of extra-pair paternity and complex patterns of cooperative breeding (ranging from non-breeding to shared reproduction) in the Seychelles warbler, the long-term inclusive fitness functions for the breeding pair of producing sons and daughters have to be

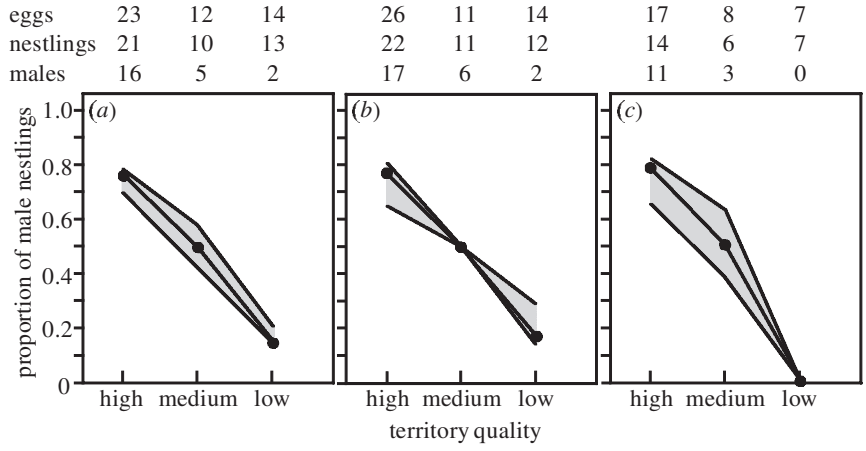

Figure 3. Sex ratio of nestlings produced by Seychelles warbler pairs in relation to quality class of breeding territory (territory quality (tq) classes: low-quality territory, medium-quality territory; and high-quality territory; 1993–1995). No additional young were present on the territory. Young were hatched from one-egg clutches only in different years. (*a*) 1993, $n = 46$, *G*-test of independence: $D = 12.23$, d.f. = 1, $p = 0.0005$, proportion of males = $1/(1 + e^{-z})$, $z = -2.68 + 1.36$ (tq class). (*b*) 1994, $n = 45$, $D = 12.03$, d.f. = 1, $p = 0.0005$, $z = -2.68 + 1.37$ (tq class). (*c*) 1995, $n = 27$, $D = 12.99$, d.f. = 1, $p = 0.0003$, $z = -3.60 + 2.10$ (tq class). Shaded areas represent the maximal and minimal values for the sex ratio assuming that all unhatched eggs were male, or female, respectively (from Komdeur *et al.* 1997).

calculated using molecular parentage analyses and precise coefficients of genetic relatedness between individuals.

5. CONCLUDING REMARKS

Although Seychelles warblers can breed independently in their first year, some individuals remain in their natal territories as subordinates, and often help providing nourishment to non-descendant offspring. The frequency of 'helping' is affected by habitat saturation and variation in territory quality. Most helpers are female, usually daughters from previous broods. However, males typically disperse. Female warblers adaptively modify the sex of their single-egg clutches according to the amount of insect food present and the number of helpers already present in the breeding territory. There is good evidence that in the long term, breeding pairs in high-quality territories obtain higher fitness by producing female offspring than male offspring, and breeding pairs in low-quality territories obtain higher fitness by producing male offspring than female offspring.

Modern kin selection theory predicts that dispersal, sex allocation and spatial population structure should strongly influence each other. However, in natural populations this interplay is far from understood because of the lack of experimental studies and accurate inclusive fitness measurements. In the future I would like to remedy this situation and to provide a rigorous test of modern kin selection theory. This is possible because the long-term study on the Seychelles warbler with extreme parental sex ratio manipulation has provided a wealth of data allowing accurate fitness measurements. Until now I have investigated the long-term benefits of sex ratio manipulation by unassisted breeding pairs based on putative pedigree data. In the future my colleagues and I will investigate the precise fitness functions of sex ratio manipulation using microsatellite markers to determine parentage and coefficients of genetic relatedness between individuals in a territory. Furthermore, we will investigate how sex-specific dispersal affects cooperation and competition between offspring, and how the balance between these forces affects decisions on sex allocation by breeding pairs with helpers already present and offspring dispersal. Limited dispersal by females means that cooperation between relatives is more likely to occur, but the same token competition over breeding opportunities is also more likely between relatives. The balance between these forces determines how parents should ideally adjust sex allocation, and how their offspring should subsequently disperse. Clearly, in the meantime, intensive fieldwork will be continued on the islands to sustain the control archive, whereas desk studies will be redoubled in joint efforts with theoretical biologists to advance the modelling studies.

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